

A-377.

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gradient from 50-600 mM NaCl. A pool was made from fractions containing >95% sHer2. sHer3 and sHer4 were also purified from conditioned media of CHO cells expressing these proteins in a similar fashion to the procedure described above. Due to its higher pI value, sHer3 was bound to and eluted from a Q-Sepharose column equilibrated in 10 mM potassium phosphate, 50 mM NaCl, pH 7.5.

EXAMPLE 2

10 Production of Anti-HER2 Antibodies

Procedures for immunizing animals, preparing fusions and screening hybridomas and purified antibodies were carried out generally as described in Harlow and Lane, 15 Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory (1988).

Enzyme-linked Immunosorbent Assay (EIA).

96-well plates were coated with 2µg/ml sHer2, 2µg/ml sHer3 or 2µg/ml sHer4 in a carbonate-bicarbonate buffer. After blocking, hybridoma conditioned medium was added to the plate and incubated for 2 hours. The medium was aspirated and the plates were washed before addition of rabbit-anti-mouse IgG antibody conjugated with horseradish peroxidase (Boehringer Mannheim). After a one hour incubation, the plates were aspirated and washed five times. Bound antibody was detected with ABTS color reagent (Kirkegaard and Perry Labs., Inc.). The extent of antibody binding was determined by monitoring the increase in absorbance at 405 nm. 30

Cloning and IgG subtype determination. Single cell cloning was done in a 96-well plate using a limiting dilution method. Conditioned media of single cell clones were screened for antibody production using the EIA 35 described above. The strongest antibody producing clones